Author’s response to reviews

Title: Influence of light-curing mode on the cytotoxicity of resin-based surface sealants.

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Author's response to reviews: see over
Dear Prof Alessandro Loguercio,

please find enclosed our revised manuscript entitled "Influence of light-curing mode on the cytotoxicity of resin-based surface sealants." for possible publication in BMC Oral Health.

Thank you to the reviewers for their valuable comments. We have taken into consideration the aspects mentioned by the referees and changed the manuscript accordingly. We hope that all corrections and changes were made in an acceptable manner. The new passages in the text are marked by yellow text-marker.

Florian J. Wegehaupt
Corresponding Author

Reviewer 1:

Remark by reviewer:
The Company/Manufacturer recommends polymerisation for 10 seconds. Furthermore, two applications and polymerisation twice is recommended. Why did you select a polymerisation time of 40 seconds as the standard?

Answer:
Sorry, this might be a miss understanding. We have set this polymerisation setting as positive control, as we assumed when starting the experiment that this group with the highest input of energy will have lowest cytotoxicity. The standard is definitive the two times 10 s polymerisation. To solve this ambiguity, we have removed the term positive control group in both the text and the figures.

Remark by reviewer:
Your study found no differences between different times and light intensity, can it therefore be inferred that the dentist can as effectively use the product with a shorter polymerisation time?

Answer:
We think to draw this conclusion by only the two studies available until now is to early. Therefore and to address your last comment, we have modified the respective part of the conclusion.

**Remark by reviewer:**
M & M: Please describe more details in the Results section; e.g. which product is better than the other.

**Answer:**
We have modified the results section, so that it gets clear, that K-0184 showed a higher cytotoxicity than Seal&Protect in the respective light-curing settings. However, these findings were not significantly.

**Remark by reviewer:**
Discussion: why is Triclosan important for cytotoxicity? You have stated the concentrations of the other ingredients. Please identify the concentration of the ingredients for each product.

**Answer:**
Unfortunately we could not get the exact concentrations, but we know from the manufacturer, that K-0184 is basically Seal&Protect but Triclosan is not included. Therefore, we assumed that the relative proportions of the remaining ingredients will change and can have an influence on the cytotoxicity. So basically not Triclosan is important for the cytotoxicity but to leave it out of the formulation has an influence (also not significant) as shown by our results. However, we have modified the respective sentence in the discussion.

**Remark by reviewer:**
Conclusion: your study concludes that the product with a shorter curing time is not toxic. However, as no information is provided regarding the abrasion and other mechanical properties, in my opinion, a recommendation cannot be given.

**Answer:**
Thank you for this comment, as stated above, we have changed this paragraph.

**Reviewer 3:**
Discretionary Revisions:

**Remarks by reviewer:**
1) At the end of the first paragraph of Background, please correct “fast”.
2) At the beginning of the first paragraph of Sample Preparation (Materials and Methods section), please correct “dentine discs were prepared form...” to “dentine discs were prepared from...”
3) Several times along the text appears “manufacture” when the correct, in those cases, is “manufacturer”.
4) In the second paragraph of Discussion, please correct “lager” for “larger”.
5) In the third paragraph of Discussion, please correct “extracellulalry” for “extracellular”.

**Answer:**
Thank you for these remarks. We have changed the manuscript accordingly.

**Minor Essential Revisions:**

**Remark by reviewer:**
1) In the Abstract, in Methods, it is important to indicate that the dentine discs are from bovine teeth. The paragraph could initiate as: “Bovine dentine discs...”

**Answer:**
This information has been added to the abstract.

**Remark by reviewer:**
2) Also in this section of Abstract, LDH must be entirely written, and not only the acronym.

**Answer:**
The whole name of LDH has been given in the abstract now.

**Remark by reviewer:**
3) Please, indicate the passage number used in the experiments, for each type of cell. This is important, mainly in the dental pulp cell culture, since there are cells that have a higher rate of proliferation and overcome the other cells. As it was not isolated a specific cell type from the dental pulps, the passage number will indicate if there were different cell types in the culture or it was a more homogeneous culture formed mainly by dental pulp fibroblasts, for example.

**Answer:**
Thank you for this point. The passages used were between 3 and 5, for both the dental pulp cells and the gingival fibroblasts. This is now indicated in the Materials and Methods section, accordingly.

**Remark by reviewer:**

4) Also, indicate the percentage of confluence that the cells were when the conditioned medium was added to the well. This is important since in a culture that have reached a 100% confluence it is expected to have a higher cell death.

**Answer:**
The cells have indeed reached 100% confluence, when the conditioned media was added to then. This is now stated in the Materials and Methods.
At this stage, a higher cell death rate is expected compared to a non-confluent cell culture. Nevertheless, the control groups can well represent the level “spontaneous” cell death in the culture, which in considerably less than when the tested material was added.

5) In Results, for each cell type, please indicate in the text which sealant showed to have a significant influence on cytotoxicity.

**Answer:**
The results sections have been changed and the information that K-0184 showed a higher cytotoxicity than Seal&Protect has been added. In the ANOVA the factor sealant showed a significant influence, however this can be due to the fact that for the control group no sealant was used.

**Remark by reviewer:**

6) It would be interesting to present, in the Discussion, the light-curing protocol and the sealant that the authors recommend for the clinician taking into account the results of this study. This is cited at the end of the Conclusion, but no protocol is really recommended, but four different protocols of light-curing that were used in the experiments.

**Answer:**
Thank you for this comment. We have added the respective protocol in the conclusion section. However, we think that it might be a bit to early to give such a
recommendation for clinical use, especially when taking in consideration the last comment given by the first reviewer.

Major Compulsory Revisions:

**Remark by reviewer:**

1) Please, indicate why the released LDH assay was chosen for the study. Why not to use an assay that would verify mitochondrial function? This would indicate that the cells were affected by the surface sealants, but not necessarily would have died. This is important since, in a clinical situation, it is expected some level of cell injury but, also, it is expected the recovery of the cells. When a cell is affected, it may release growth factors that will attract other cells and help to mediate and resolve the inflammatory process started by the injury, which in the case of the manuscript would be the cytotoxicity of the sealants, but not necessarily the cell has to die.

**Answer:**

We understand the reviewer’s concern. A mitochondrial-based assay could also have been used (such as MTT), but we have chosen the LDH activity assay, as it is a very well established and highly reproducible assay. We sought to identify clear cytotoxicity, identified by cell lysis and release of the endoplasmic content of the cell into the environment. Certainly different material may not have a direct “lytic” effect, but could trigger cell damage and repair. Nevertheless, this would be a largely mechanistic question of cell biology, which would require more specific assays that measure apoptosis. Since this would have been beyond the scope of our study, we preferred to utilize a more “definitive” cytotoxicity assay, which in this case is the measurement of the released LDH activity. This was already considered, but now further reinforced, in the second paragraph of the Discussion section.

**Remark by reviewer:**

2) Explain why it was not included a naïve control group (cells cultured in regular media). It would help to eliminate any factor present in the conditioned media that could eventually have been released from the bovine dentine. With this information, data could be more accurate regarding the influence of the surface sealants on cytotoxicity and cell death.

**Answer:**
The naïve control group is indeed included in the Figures (left side bar, as untreated control). This clearly indicates that the “natural” cell death in the culture is at the range of 10% for both cell types, which is considerably lower than what is seen with the tested material.

Editorial comments:
1) Please clarify the scientific advance that your article provides over previous publications included in your references (18-20).

Answer:
Thank you for this comment. The references 18-20 deal with the influence of the degree of conversion on the cytotoxicity of resin-based materials. Our study aimed to investigate if the negative side effect of a shortened light-curing duration (increased cytotoxicity) can be compensated by a simultaneously increased light intensity while light curing surface sealants. This approach makes for us the novelty of our study as to our best knowledge, no other study has ever tried this.

To point out the novelty of our study, we have added the following paragraph: “To the present, no study has been published that examines the use of increased light intensity to compensate for the negative side effects (increased cytotoxicity) of a shortened light-curing duration of surface sealants. If this shortening is possible without negative effects (this means increased cytotoxicity), this might reduce the time consumed in the procedure, when sealing is applied in whole dentition or numerous teeth.”

2) Please proof read your manuscript.

Answer:
Thank you for this valuable comment. We have had the manuscript checked by a native English speaker.